BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Amendments to the Specification:

Please replace paragraph **[0010]** with the following amended paragraph:

[0010] In accordance with the purpose of the invention as embodied and broadly described herein, a method for detecting the fungus *Stachybotrys chartarum* includes isolating DNA from a sample suspected of containing the fungus *Stachybotrys chartarum*; subjecting the DNA to polymerase chain reaction amplification utilizing at least one primer, wherein the at least one primer comprises one of a (~~SEQ. ID NO.~~ SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3', (~~SEQ. ID NO.~~ SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3', (~~SEQ. ID NO.~~ SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3', and (~~SEQ. ID NO.~~ SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3' base sequence; and detecting the fungus *Stachybotrys chartarum* by visualizing the product of the polymerase chain reaction.

Please replace paragraph **[0011]** with the following amended paragraph:

[0011] In another exemplary embodiment consistent with the invention, a primer set for detecting *Stachybotrys chartarum* using polymerase chain reaction includes a first primer comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3'; and a second primer comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3'.

Please replace paragraph **[0012]** with the following amended paragraph:

[0012] In a further exemplary embodiment consistent with the invention, a primer set for detecting *Stachybotrys chartarum* using polymerase chain reaction includes a first primer comprising a first base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3'; and a second primer comprising a second base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3'.

Please replace paragraph **[0013]** with the following amended paragraph:

[0013] In an additional exemplary embodiment consistent with the invention, a primer and probe set for detecting the fungus *Stachybotrys chartarum* using polymerase chain reaction includes a forward primer comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3'; a reverse primer comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3'; and a probe comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 5) 6-FAM-5'CTGCGCCCGGATCCAGGC3'-TAMRA.

Please replace paragraph **[0014]** with the following amended paragraph:

[0014] In another exemplary embodiment consistent with the invention, a primer and probe set for detecting the fungus *Stachybotrys chartarum* using polymerase chain reaction, includes a forward primer comprising a first base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 3)

5'ACCTATCGTTGCTTCGGCG3'; a reverse primer comprising a second base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3'; and a probe comprising a base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 5) 6-FAM-5'CTGCGCCCGGATCCAGGC3'-TAMRA.

Please replace paragraph **[0015]** with the following amended paragraph:

[0015] In a further exemplary embodiment consistent with the invention, a method for detecting the presence of the fungus *Stachybotrys chartarum* includes obtaining a sample from the environment; extracting DNA from the sample; and amplifying the extracted DNA by polymerase chain reaction utilizing one or more primers to obtain an indication of the presence of *Stachybotrys chartarum* in the sample, wherein the one or more primers comprise at least one of a (~~SEQ. ID NO.~~ SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3', (~~SEQ. ID NO.~~ SEQ ID NO: 2) 5'TTTGCGTTTGCCACTCAGAG3', (~~SEQ. ID NO.~~ SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3', and (~~SEQ. ID NO.~~ SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3' base sequence.

Please replace paragraph **[0016]** with the following amended paragraph:

[0016] In yet another exemplary embodiment consistent with the present invention, a method for detecting the presence of the fungus *Stachybotrys chartarum* includes obtaining a sample from the environment; extracting DNA from the sample; and amplifying the extracted DNA by polymerase chain reaction utilizing a primer set to obtain an indication of the

presence of *Stachybotrys chartarum* in the sample, wherein the primer set comprises: a forward primer comprising a first base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3', and a reverse primer comprising a second base sequence (~~SEQ. ID NO.~~ SEQ ID NO: 4) 5'GCGTTTGCCACTCAGAGAATACT3'.

Please replace paragraph **[0037]** with the following amended paragraph:

[0037] After concentration and purification of the spore suspension DNA, a minimum of four serially diluted concentrations of standards, in duplicate with replicate unknown samples, may be amplified by polymerase chain reaction. The ABI Prism 7700 Sequence Detection System (7700 SDS; Applied Biosystems, Foster City, CA) may be used, for example (step 710). Amplification conditions using, for example, Applied Biosystems reagents may include the following:

fungal DNA template (5µl)

1 X TaqMan™ buffer A

5 mM $MgCl_2$

0.1 mM dATP

0.1 mM dCTP

0.1 mM dGTP

0.2 mM dUTP

2.5 U AmpliTaq Gold

0.5 U AmpErase Uracyl N-Glycosylase

0.9 µM of each of the forward and reverse *S. chartarum* primers from either selected

primer set:

Primer set 1:

forward (STAF1) primer: (~~SEQ. ID NO.~~ SEQ ID NO: 1) 5'GTTGCTTCGGCGGGAAC3'
reverse (STAR1) primer: (~~SEQ. ID NO.~~ SEQ ID NO: 2)
5'TTTGCGTTTGCCACTCAGAG3'

Primer set 2:

forward (STAF2) primer: (~~SEQ. ID NO.~~ SEQ ID NO: 3) 5'ACCTATCGTTGCTTCGGCG3'
reverse (STAR2) primer: (~~SEQ. ID NO.~~ SEQ ID NO: 4)
5'GCGTTTGCCACTCAGAGAATACT3'

0.2 μM of the following *S. chartarum* probe:

(~~SEQ. ID NO.~~ SEQ ID NO: 5) 6-FAM-5'CTGCGCCCGGATCCAGGC3'-TAMRA

1X Exogenous Internal Positive Control mix (IPC with VIC™-labeled probe, Applied Biosystems)

1X IPC DNA

for a total reaction volume of 50μl. TaqMan™ cycling conditions may include the following: 2 minutes at 50° C; 10 minutes at 95° C; 40 cycles of 15 seconds at 95° C followed by 1 minute at 60° C.

Please replace paragraph **[0041]** with the following amended paragraph:

[0041] Two methods were tested for the removal of spore and/or hyphal fragments from spore suspensions. One cleaning method consisted of filtration of the *S. chartarum* spore suspension through 2 or 3 μm membranes (Millipore Corp., Bedford, MA, USA). Membranes tested were: mixed cellulose ester, 3 μm; polycarbonate, 2 and 3 μm; and teflon, 3 μm. Sucrose density centrifugation was also tested for the removal of spore fragments.

One ml of spore suspension was added to approximately 25 ml of filter-sterilized sucrose (0.35 M) and centrifuged at 1000 x g for 10 min. at room temperature (Wang, NS[[,]] ~~website, http://www.eng.umd.edu/~nsw/ench485/lab10.htm~~). The supernatant was removed without disturbing the pellet. The spore pellet with residual sucrose was resuspended in 200 µl PBT and stored at 4° C overnight to settle. Cloudy supernatants above spore pellets were removed and the spore pellet was resuspended in PBT as before. Effectiveness of the cleaning methods was assessed by enumeration of the spore suspensions with an electronic particle counter (see PCR quantitation standards and analysis section). When necessary, a second sucrose centrifugation was performed using 0.70 M sucrose. Clean spore suspensions were stored at –70° C until ready for use.